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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:
Schmitt *et al.*

SERIAL NO.: 10/822,222

FILED: April 9, 2004

FOR: **Enzymatic Modification of Lecithin**

EXAMINER: Allison M. Ford

ART UNIT: 1651

CONF. NO.: 1315

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Dr. Michael Schneider, declare and affirm as follows:

I currently hold the position of R&D consultant at Cargill Inc., where I have been employed since May 2004.

I received a PhD from Hamburg University in 1976 in the field of Chemistry.

I have worked with the inventors and the assignee of the above-referenced application, on a consulting basis, since May 2004.

I have read and am familiar with the subject matter of the above-referenced application, the pending claims, and the cited reference, Sas *et al.*, "Method for the Conversion of Lecithin in Lysolecithin" (U.S. Patent No. 6,068,997, equivalent to PCT Pubn No. WO 2000/52190), which has been cited as prior art against these claims.

Claims 1 and 18 in the application are directed to processes in which a starting material containing phospholipids and triglycerides (e.g., a crude lecithin) is hydrolyzed with both a lipase and a phospholipase, either in an aqueous solvent (containing less than 5% of an additional water miscible solvent, and preferably no additional solvent) or in an aprotic organic solvent, such as hexane.

I understand that these claims and/or their dependent claims have been rejected on the grounds that their subject matter would have been obvious in view of Sas *et al.* and other references. Of these, Sas *et al.* is the only reference claiming to describe the hydrolysis of a starting material containing phospholipids and triglycerides with both a lipase and a phospholipase.

In my view, the process described by Sas *et al.* differs in at least the following significant respects from the processes of the claims:

1. The Sas *et al.* patent states that triglycerides in the starting material are hydrolyzed to mono/diglycerides by the lipase. However, the process described in Sas *et al.* employs, as the reaction medium, a 6:1 mixture of water and glycerol. The glycerol would be expected to react, at least in part, with the free fatty acids released upon hydrolysis of the phospholipid molecules. While a fraction of the mono/diglycerides formed in Sas *et al.* may come from triglyceride hydrolysis, a large portion would be products of transesterification, in view of the large quantity of glycerol present.

Therefore, a very different product ratio would be expected if the glycerol in the reaction mixture described in Sas *et al.* were absent or significantly reduced, as in the claimed processes.

2. When hydrolysis of crude lecithin is carried out in water, the phospholipids are generally present in micellar form. Addition of glycerol, especially in the amount described in Sas *et al.*, would have the effect of solubilizing the micellar structures, giving, most probably, better access to the molecules for the hydrolyzing enzyme.

The presence of glycerol or other polyols is also known to affect the activity of enzymes. For example, Falcone *et al.*, 2004, enclosed, describes an increase in activity of α-chymotrypsin with increased glycerol in a glycerol/water system. They state that "the efficiency of the enzymatic reaction in homogeneous media is nearly 8 times greater in the GY-water mixture than in water, probably as a result of the high viscosity around the enzyme in the organic mixture and, consequently, the lower mobility of the protein" (p 5736, 3rd full paragraph). Addition of glycerol to a reverse micellar solution in water/surfactant/heptane gave a similar result: "Comparing the catalytic efficiencies obtained in both reverse micellar systems, GY-water/AOT/n-heptane and water/AOT/n-heptane, it can be seen that the value in the former

one is 5 times higher. This fact can also be explained by considering that the addition of GY in the reverse micellar aggregates results in a decrease of conformational mobility of the α-CT..., which leads to an increase of the enzyme stability and activity" (p. 5737, 3rd paragraph).

Earlier publications show that the effect of glycerol and other polyols on protein structure and conformation in aqueous systems was well known in the art, and was believed to apply to proteins in general. See, for example, Gekko and Timasheff, 1981, also enclosed.

It is likely that such solvent effects are at least partly responsible for the high conversion of phospholipids to lysophospholipids described by Sas *et al.* The Sas *et al.* patent places great emphasis on these high conversion rates. For example, the patent discloses that over 90% conversion was obtained, vs. about 10-20% for the prior art comparative data (as shown in Tables 1-2 of the patent).

Because it is very likely that the presence of the polyol co-solvent contributes to this high conversion rate, one attempting to reproduce this effect would not have any reason to remove the glycerol from the reaction system. Therefore, the teachings of Sas *et al.* differ significantly from the processes of the pending claims.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

06/02/2006
Date

Michael Schneider, Ph.D.